PCT WORLD INTELLECTUAL Internati

INTERNATIONAL APPLICATION PUBLISHED UI

(51) International Patent Classification 6 : C07K 14/57, 16/24

A2

(11) International Publication Number: WO 96/03435

(43) International Publication Date: 8 February 1996 (08.02.96)

(21) International Application Number: PCT/GB95/01731

(22) International Filing Date: 21 July 1995 (21.07.95)

(30) Priority Data:
9414752.7 21 July 1994 (21.07.94) GB

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(81) Designated States: AU, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: FELINE γ-INTERFERON

CTACTGATTTCAACTTCTTTGGCCTAACTCTCCGAAACGATGAATTACACAAGTTTTATT
* L S E T M N Y T S F I

TTCGCTTTCCAGCTTTGCATAATTTTGTGTTCTTCTGGTTATTACTGTCAGGCCATGTTT
F A F Q L C I I L C S S G Y Y C Q A M F

TTTAAAGAAATAGAAGAGCTAATGGGATATTTTAATGCAAGTAATCCAGATGTAGCAGAT
F K E I E E L M G Y F N A S N P D V A D

GGTGGGTCGCTTTTCGTAGACATTTTGAAGAACTGGAAAGAGGAGAGTGATAAAACAATA
G G S L F V D I L K N W K E E S D K T I

ATTCAAAGCCAAATTGTCTCCTTCTACCTGAAAATGTTTGAAAACCTGAAAGATGATGAC
I Q S Q I V S F Y L K M F E N L K D D D

CAGCGCATTCAAAGGAGCATGGACACCATCAAGGAAGACATGCTTGATAAGTTGTTAAAT
Q R I Q R S M D T I K E D M L D K L L N

ACCAGCTCCAGTAAACGGGATGACTTCCTCAAGCTGATTCAAATCCCTGTGAATGATCTG
T S S S K R D D F L K L I Q I P V N D L

CAGGTCCAGCGCAAAGCAATAAATGAACTCTTCAAAGTGATGAATGATCTCTCACCAAGA
Q V Q R K A I N E L F K V M N D L S P R

TCTAACCTGAGGAAGCGGAAAAGGAGCCAGAATCTGTTTCGAGGCCGTAGAGCATCGAAA
S N L R K R K R S Q N L F R G R R A S K

TAATGGTTGTCCTGCCTGCAATATTTG
*

(57) Abstract

The present invention provides a polynucleotide fragment encoding feline γ-interferon, a recombinant vector comprising such a polynucleotide fragment, a host cell containing said polynucleotide fragment or recombinant vector, a recombinant feline γ-interferon polypeptide, and pharmaceutical compositions comprising recombinant γ-interferon for use as a prophylactic and/or therapeutic agent and also as an adjuvant.

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FELINE γ-INTERFERON

The present invention relates to feline γ-interferon, a polynucleotide fragment encoding feline γ-interferon, a recombinant vector comprising such a polynucleotide fragment, a host cell containing said polynucleotide fragment, or recombinant vector comprising said polynucleotide fragment, a feline γ-interferon polypeptide, antibodies immuno-reactive with said polypeptide and pharmaceutical compositions comprising recombinant feline γ-interferon for use as a prophylactic and/or therapeutic agent and also as an adjuvant in cats.

Cytokines are low molecular weight secreted proteins with immunomodulatory activity. The term applies to interferons, interleukins, lymphokines, monokines, colony stimulating factors and a number of growth factors (Balkwill and Burke 1989). Interferons are a class of cytokines which exhibit antiviral activity. Three types exist, alph (α), beta (ß) and gamma (γ) (Farrar and Schreiber 1993). The type I interferons, α and ß are related and bind to a common cell structure surface receptor, although the multiple forms of α and the single ß form may have different biological effects. Interferon gamma (IFNγ), also known as type II or immune interferon, is distinct from α and ß interferons at a genetic level. Like ß interferon it is encoded by a single gene. IFNγ is

produced by natural killer (NK) cells and by T lymphocytes (TH1 subset) in response to stimulation with antigen or mitogens. It possesses a range of biological effects of immunomodulatory or antiviral nature including enhancement of MHC class I and class II expression, suppression of the TH2 T lymphocyte response, activation of macrophages, enhancement of natural killer cell activity, and modulation of the synthesis and effect of a number of cytokines (Gray 1992). IFNγ has also exhibited adjuvanticity properties in some animal models (reviewed in Health and Playfair 1992).

Although the bovine and ovine IFNγs, which exhibit 93% amino acid identity, are cross-reactive (Radford et al, 1991), IFNγs tend to be highly species-specific in their biological activity. Thus investigation of the potential applications of IFNγ in a particular species generally requires production of recombinant IFNγ of that species. Since the goals of most interferon cloning operations are deduction of the amino acid sequence and production of recombinant IFN, most IFNγ sequences have been derived from mRNA rather than chromosomal DNA. The advent of the polymerase chain reaction has, however, facilitated the cloning of cytokine cDNAs and the nucleotide sequences of the genes or cDNAs encoding a number of mammalian cytokines to been determined.

The most extensive structural studies of IFNγ have been performed on the human cytokine. The human IFNγ gene is 6kbp in size and possesses 3 introns (Taya et al, 1982). The human IFNγ gene encodes a 166 amino acid pr cursor

possessing a 23 amino acid N-terminal signal sequence and two N-linked glycosylation sites. Functional IFNγ is homodimeric with the polypeptides associated in a non-covalent manner (Scahill et al, 1983). Several forms of human IFNγ exist due to post-translational C-terminal proteolytic cleavage and differential glycosylation (Rinderknecht et al, 1984). Regions of N-terminal and C-terminal sequence have been determined as being important in maintaining biological activity (Farrar and Schreiber, 1993).

Cytokine and anti-cytokine therapies such as synthesis of inhibitors, soluble cytokine receptors, receptor antagonists or anti-cytokine antibodies, are finding increased clinical application in human medicine (Mire-Sluis, 1993). Due to the lack of species cross-reactivity of many cytokines and the potential for antibody production against heterologous cytokines (Holmes, 1993), however, the ideal reagents in the cat will be substantially feline-specific.

The present invention provides a polynucleotide fragment, such as a DNA fragment, encoding feline γ-interferon. The invention further provides a recombinant feline γ-interferon polypeptide.

"Polynucleotide fragment" as used herein refers to a chain of nucleotides such as deoxyribose nucleic acid (DNA) sequences and transcription products thereof, such as RNA capable of giving rise to a feline γ-interferon protein or physiologically active fragments thereof. Thus, this term

includes double and single stranded DNA, and RNA sequences derived therefrom. The term excludes the whole naturally occurring genome.

Generally, the polynucleotide will be in isolated form substantially free of biological material with which the whole genome is normally associated in vivo.

In general, the term "polypeptide" refers to a chain or sequence of amino acids displaying a biological activity substantially similar to the biological activity of feline γ-interferon and does not refer to a specific length of the product as such. The polypeptide, if required, can be modified in vivo and/or in vitro, for example by glycosylation, amidation, carboxylation, phosphorylation and/or post translational cleavage thus inter alia peptides, oligopeptides and proteins are encompassed thereby.

The DNA fragment encoding feline γ-interferon can be amplified from feline γ-interferon cDNA obtained by way of reverse transcription of mRNA by polymerase chain reaction (PCR) techniques known in the art, using primers designed against conserved regions of γ-interferon coding sequences from a single species, or a number of other species. An amplified fragment containing the feline γ-interferon is depicted in Figure 1.

The DNA fragment of Figure 1 was shown to encode an open reading frame (ORF) of 171 amino acids. A comparison of the amino acid sequence against previously sequenced γ-interferons rev aled identities ranging from 68 % with

murine γ-interferon to 78% with human γ-interferon. The two potential N-linked glycosylation sites exist at positions conserved in bovine and pig γ-inteferon precursors at amino acids 39-41 and 107-109 in the feline precursor (data not shown). In common with the human γ-interferon, feline γ-interferon has a predicted N-terminal signal sequence and possesses no cysteine residues.

The present invention includes polynucleotide and polypeptide sequences having at least 80 %, particularly at least 90 % and especially at least 95 % similarity with the sequences of Figure 1. "Similarity" refers to both identical and conservative replacement of nucelotides or amino acids, provided that the functionality of feline γ-interferon is substantially unimpaired.

The skilled man will appreciate that it is possible to genetically manipulate the gene or derivatives thereof, for example to clone the gene by recombinant DNA techniques generally known in the art and to express the polypeptides encoded thereby in vitro or in vivo. Polynucleotide fragments having the nucleotide sequence depicted in Figure 1 or derivatives thereof are preferably used for the expression of the feline γ-interferon.

It will be understood that for the particular feline γ-interferon polypeptide embraced herein, natural variations can exist between individuals or between species of the felis genus. These variations may be demonstrated by (an) amino acid difference(s) in the overall sequence or by deletions, substitutions,

insertions, inversions or additions of (an) amino acid(s) in said sequence. All such derivatives showing active feline γ-interferon physiological activity are included within the scope of this invention. For example, for the purposes of the present invention conservative replacements may be made between amino acids within the following groups:

(i) Alanine, Serine, Threonine;

(ii) Glutamic acid and Aspartic acid;

(iii) Arginine and Lysine;

(iv) Asparagine and Glutamine;

(v) Isoleucine, Leucine, and Valine;

(vi) Phenylalanine, Tyrosine and Tryptophan.

Moreover, recombinant DNA technology may be used to prepare nucleic acid sequences encoding the various derivatives outlined above.

As is well known in the art, the degeneracy of the genetic code permits substitution of bases in a codon resulting in a different codon which is still capable of coding for the same amino acid, e.g. the codon for the amino acid glutamic acid is both GAT and GAA. Consequently, it is clear that for the expression of a polypeptide with the amino acid sequence shown in Figure 1 or fragment thereof use can be made of a derivative nucleic acid sequence with such an alternative codon composition differ nt from the nucleic acid sequence shown in said

Figure 1.

Furthermore, fragments derived from the feline γ-interferon polypeptide or from the amino acid sequence depicted in Figure 1 which still display feline γ-interferon properties, or fragments derived from the nucleotide sequence encoding the feline γ-interferon polypeptide or derived from the nucleotide sequence depicted in Figure 1 encoding fragments of said feline

γ-interferon polypeptides are also included in the present invention.

All such modifications mentioned above resulting in such derivatives of the feline γ-interferon polypeptide or gene are covered by the present invention so long as the characteristic feline γ-interferon biological properties remain substantially unaffected.

The feline γ-interferon polynucleotide fragment of the present invention is preferably linked to regulatory control sequences. Such control sequences may comprise promoters, operators, inducers, ribosome binding sites terminators etc. Suitable control sequences for a given host may be selected by those of ordinary skill in the art. In particular, a feline γ-interferon control sequence can be employed in a mammalian host.

A polynucleotide fragment according to the present invention can be ligated to various expression controlling polynucleotide sequences, resulting in a so-called recombinant nucleic acid molecule. Thus the present

invention also includes an expression vector containing an expressible nucleic acid molecule. Said recombinant nucleic acid molecule can then be used for transformation of a suitable host. Such hybrid polynucleotide molecules are preferably derived from for example plasmids, or from nucleic acid sequences present in bacteriophages or viruses and are termed vector molecules.

Specific vectors which can be used to clone nucleic acid sequences according to the invention are known in the art (e.g. Rodriguez, R.L. and D.T. Denhardt, edit., Vectors: A survey of molecular cloning vectors and their uses, Butterworths, 1988).

A specific bacterial expression vector pHEX has been adapted for γ-interferon production (Reid, submitted for publication). The pHEX vector has a stretch of 6 histidine residues and a thrombin site downstream of the vector-specified ATG initiation codon. The 6 histidine residues provide crude purification of recombinant proteins using affinity chromatography. The DNA insertion site is positioned 3′ to the thrombin site so that should biological activity of the feline γ-interferon produced be compromised by the vector-specified N-terminus, cleavage of extraneous amino-acid sequence from the interferon moiety is possible.

The methods to be used for the construction of a recombinant nucleic acid molecule according to the invention are known to those of ordinary skill in the art and are inter alia set forth in Sambrook, et al. (Molecular

Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, 1989).

The present invention also relates to a transformed cell containing the feline γ-interferon polynucleotide fragment in expressible form. "Transformation", as used herein, refers to the introduction of a heterologous polynucleotide fragment into a host cell, irrespective of the method used, for example direct uptake, transfection or transduction. The heterologous polynucleotide fragment may be maintained through autonomous replication or alternatively, may be integrated into the host genome. The recombinant nucleic acid molecules preferably are provided with appropriate control sequences compatible with the designated host which can regulate the expression of the inserted polynucleotide fragment, e.g. tetracycline responsive promoter, thymidine kinase promoter and SV-40 promoter.

Suitable hosts for the expression of recombinant nucleic acid molecules can be prokaryotic or eukaryotic in origin. The most widely used hosts for expression of recombinant nucleic acid molecules may be selected from bacteria, yeast, insect cells and mammalian cells.

Since the biological half-life and the degree of glycosylation of recombinant feline γ-interferon may be important for use in vivo, yeast and baculovirus systems are preferred. The yeast strain Pichia pastoris exhibits potential for high level expression of recombinant proteins (Clare et al,1991). The baculovirus system has been used

successfully in the production of type 1 interferons (Smith et al, 1983), and by the present invention for the production of feline γ-interferon.

The cloning and expression of recombinant feline γ-interferon also facilitates in producing reagents for the production of, for example, probes for in situ expression studies, production of anti-feline γ-interferon antibodies (particularly monoclonal antibodies) and evaluation of in vitro and in vivo biological activity of recombinant feline γ-interferon. The antibodies may be employed in diagnostic tests for γ-interferon.

The present invention further provides recombinant feline γ-interferon for the manufacture of reagents for use as prophylactic and/or therapeutic agents and also as an adjuvant in cats. In particular the invention provides pharmaceutical compositions comprising the recombinant γ-interferon together with a pharmaceutically acceptable carrier therefor.

A wide range of feline abnormalities such as disease states attributable to feline γ-interferon deficiencies or over abundance and physiological or immunological abnormalities could benefit from feline γ-interferon therapy and/or prophylaxis. Such disease states include cancer, endotoxaemia, parasitic and bacterial infections, wound therapy, auto-immune and inflammatory diseases, allergies and viral infections. Feline γ-interferon may also be applied for vaccine adjuvantation.

Cytokines such as interferons α, β and γ and tumour

necrosis factor, TNF, are reported as being capable of exhibiting direct antiviral activity in addition to their immunomodulatory properties (Ramsay et al, 1993). The recombinant feline γ-interferon can prove beneficial as a prophylactic agent to modulate recrudescence of viruses, such as feline herpesvirus (FHV), under conditions of stress and to reduce the clinical consequence of horizontal transmission of viral agents to in-contact animals. Herpes viruses and other viruses with complex genomes play an immunomodulatory role contributing to general or specific immunosuppression in the host. Administration of the feline γ-interferon can also serve to redirect the immune response such that more efficient virus clearance occurs. By way of example, administration of bovine IFNγ prior to experimental infection of cattle with bovine herpesvirus-1 (BHV-1) has been known to reduces virus-induced suppression of cell-mediated cytotoxicity (Bielefeldt Ohmann and Babiuk, 1984).

As a consequence of the pleiotropic nature of the cytokine system, administration of the recombinant feline γ-interferon may have consequences on the expression and biological effect of other cytokines. Thus feline γ-interferon can be used as a tool to study these effects.

Non-specific stimulation of the immune response by adjuvant formulations is often beneficial in cases of prophylactic or therapeutic vaccination. However, in particular instances a more defined stimulation of the immune response using feline γ-interferon as an adjuvant or

co-adjuvant can be more effective. Such a stimulation could take the form of administration of recombinant feline γ-interferon or expression of feline γ-interferon within live or disabled virus vectors. γ-interferon has previously been reported as being associated with adjuvancy in animals (Heath and Playfair, 1992). IFNγ upregulate MHC class II expression enhancing antigen presentation, and suppresses TH2 lymphocytes with resultant inhibition of humoral immunity. Through such actions an IFNγ adjuvant can drive a vaccine-protective immunity against viruses (Ramshaw et al, 1992). Feline γ-interferon may also ameliorate clinical response to live virus vaccination which, providing protective immunity is not compromised, could be a desirable safety feature (Andrew et al, 1991; Ramshaw et al, 1992). Candidate live virus vectors include poxviruses, adenovirus and herpesvirus, though the latent capacity of the latter coupled with potential immunopathology of cytokines may preclude cytokine insertion in this virus.

Administration of feline γ-interferon with live feline herpesvirus vaccines may be used to overcome mechanisms of immune evasion or diversion specified by the virus to provide a longer lasting protective immune response.

Embodiments of the invention will now be described by way of example only.

EXAMPLE 1

Molecular cloning of the feline γ-Interferon cDNA

mRNA was isolated from mitogen-stimulated feline lymphocytes using the RNAgents (Trademark) Total RNA isolation system (supplied by Promega Corp.) 5μg of the mRNA was converted to cDNA using the Superscript (Trademark) system (supplied by Life Technologies).

Two oligonucleotides (see below) were designed against interspecies conserved non-coding regions of γ- interferon mRNA.

Upstream primer (I):- 5'- CTACTGATTTCAACTTCTTTGC-3'

Downstream primer (II):- 5'- CAAATATTGCAGGCAGGACAACC-3'

These oligonucleotides were then used as primers in a polymerase chain reaction (PCR) experiment to amplify the feline γ-interferon cDNA.

The PCR was performed as described by Saiki et al (1987). Ten μl of 100ng/μl template cDNA from the reverse transcribed mRNA was added to a 40μl reaction mixture containing 200μM of dATP, dCTP, of dGTP and dTTP, 50pmol of both primers (I) and (II), 1 unit of DNA Polymerase and 5μl of 10x reaction buffer. The reaction buffer contained 100mM Tris-HCl, 500mM potassium chloride, 0.01 per cent (w/v) gelatin and 1.5mM magnesium chloride, ultrapure water, TE (pH8.0). The solution was overlaid with two drops of mineral oil to prevent evaporation. To eliminate the possibility of false positives from the contamination of genomic samples with preparation of cDNA, amplification

and analysis of the products were carried out. Thirty five cylces of amplification were performed using a Perkin Elmer Cetus thermal cycler. Each cycle consisted of 1 min. at 95°C to denature the DNA, 1 min. at 50°C to anneal the primers to the template and 1 min. at 72°C for primer extension. After the last cycle a further incubation for 10mins. at 72°C was performed to allow extension of any partially completed product. On completion of the amplification, 10µl of the reaction mixture was electrophoresed through a 1.5 per cent agarose gel. The DNA was visualised by staining with ethidium bromide and exposure to ultraviolet light (320nm).

An amplified reaction product of approximately 600 bp was observed and the DNA purified and cloned into the phagemid vector PCR-Script (Trademark) sk (+) (supplied by Stratagene).

EXAMPLE 2

Sequencing of the cloned feline γ-interferon cDNA

The PCR-Script (Trademark) derived vector, containing the cloned feline γ-interferon DNA was prepared and purified. Double stranded DNA sequencing was carried out on this DNA using the T7 polymerase sequenase (Trademark) kit (supplied by USB Corp.) as per the manufacturers instructions.

The DNA sequence obtained is shown in Figure 1. The two underlined regions at the ends of the sequence correspond to the two primers (I) and (II) used to amplify the DNA. The

two internal underlined regions represent potential glycosylation sites.

A translation of the DNA sequence into its corresponding amino acid sequence (one-letter code) is shown below the DNA sequence.

EXAMPLE 3

Expression of recombinant feline γ-interferon in Escherichia coli.

The open reading frame (ORF) encoding the feline γ-interferon was excised from the PCR-Script (TRADEMARK) derived vector and sub-cloned into the plasmid vector pHEX (Reid, 1994 submitted) for expression.

The pHEX vector was constructed from the vector pGEX-2T (Smith and Johnson, 1988). The sequence of the glutathione S-transferase gene was deleted from pGEX-2T and a sequence which contained a consensus to the 5′ coding regions of bacterial mRNA and also six histidine residues was inserted 3′ to the initiating AUG (see below)

pHEX BamHI EcoRI

Met Ala Lys Ile Asn His His His His His His Gly Ser Glu Phe

tac>ATG GCT AAA ATA AAT CAT CAC CAT CAC CAT CAC GGA TCC GAA TTC

On expression of the cloned feline γ-interferon DNA, a polypeptide of approximately 20 kilodaltons was observed, when crude lysates of IPTG induced cultures were analysed on a 12%

acrylamide gel (Laemmli, 1970), followed by staining with coomasie brilliant blue dye.

EXAMPLE 4

Expression of Feline γ-interferon in baculovirus system

Feline γ-interferon cDNA, previously cloned in the pCR script ™ SK⁺ plasmid vector was excised on SstI and BamHI and cloned into similarly digested pAcCL 29-1 (Kang, 1988) which was then used to transform E.coli INVαF' (invitrogen). A stock of the feline γ-interferon containing transfer vector was then prepared using conventional techniques.

Wild type Autographa californica Nuclear Polyhedrosis virus (AcMNPV) was grown up in Sf9 cells and infectious virus DNA purified by caesium gradient and its concentration determined.

1μg of the above wild-type DNA was included in two restriction enzyme digestion reactions. Each reaction included 150μl of viral DNA, 18μl of buffer #3 (Life Sciences), 2μul of Bsu36I, 1.8μl of acetylated BSA, and 8.2μl of water to give a final volume of 180μl. The reaction was digested overnight at 370°C.

Co-Transfection of Insect Cells

Co-transfection of insect cells with linearized virus and transfer vector was achieved using the calcium phosphate co-precipitation method. To facilitate the success of this procedure, a standard calcium phosphate co-precipitation kit (Stratagene) was used. This kit employs two solutions, #1 and #2. #1 is 2.5M $CaCl_2$ and #2 is 2xBBS (pH 6.95) consisting of

50mM N,N-bis (2-hydroxyethyl)-2-aminoethanesulfonic acid and buffered saline, 280mM NaCl and 1.5mM Na_2HPO_4.

Dishes containing 10^6 Sf9 cells were prepared and allowed to settle for around 2-3 hours. Three co-transfection reactions were set up, namely, linearized viral DNA + Transfer vector, linearized viral DNA alone and uncut wild-type virus alone, the latter two acting as controls. The reactions were set up as follows: 1μg of linearized viral DNA and 5μg of transfer vector were diluted with distilled, deionized water to 450μl to which was added 50μl of solution #1. 500μl of solution #2 was added slowly and the solutions mixed gently. The mixture was allowed to incubate at room temperature for 20 minutes. After incubation the medium was removed from the 35mm dishes and 1ml of each co-transfection mixture added to the appropriate plates. The cells were incubated for 1 hour at room temperature. Following this incubation, a further 1ml of TC 100 medium was added to the plates, which were then allowed to incubate overnight at 28°C. The following day, the medium was removed and the monolayer washed with calcium free phosphate buffered saline. Two ml of medium was then added to each plate and the plates allowed to incubate at 28°C for a further 48 hours. After 48 hours the co-transfection mixtures were harvested, filtered through a 0.45uM filter and stored at 40°C. The next step was to separate recombinant and wild-type virus.

Separation of Parental & Recombinant Viruses by Plaque Assay

The required number of 35mm dishes were prepared, as

described, using 10^6 Sf9 cells/dish. Dilutions of the co-transfection mixtures were then made in TC100 growth medium, viz: neat, 10^{-1}, 10^{-2} and 10^{-3}. The medium from the Sf9 cells was removed and 100µl of the appropriate dilution was added to the appropriate dish. The dishes were allowed to incubate at room temperature for 1 hour. Following this incubation step, the inoculum was removed and replaced with a 2ml agarose/TC100 overlay. The dishes were then incubated at 28°C for 3 days and then stained in the usual way, using neutral red and X-gal. A number of recombinant plaques were observed and one was used for subsequent experiments.

Production of Recombinant Protein

The protocol consisted of infecting cells which were in logarithmic growth (cell viability>98%) and at a concentration of $5x10^5$ cells per ml. For the production of the first batch of feline γ-interferon protein, $1x10^7$ Sf9 cells were used to seed 100ml of TC100 medium in a 500ml spinner culture flask. The cells were allowed to go through two doublings before being counted and subsequently infected with recombinant virus. The cells were infected when they had reached a concentration of $5x10^5$ cells/ml (after 48 hours) and the infecting dose of virus used was 10 pfu/cell. The supernatant was harvested 5 days post infection and was subjected to centrifugation (1500 rpm/10mins) to remove cell debris. The product was then filtered through a 0.2uM filter and stored at -20°C. This first batch of crude interferon protein was designated DA1 and was subsequently used in the bioassays described below.

In addition to the production of DA1, a second batch of crude interferon was produced, designated DA2. The conditions for production were as described above but the protein was produced by the infection of High Five (invitrogen) insect cells. These cells are reported to increase protein production 10 fold.

EXAMPLE 5

Cytopathic Reduction Assay using Calicivirus Infected Feline Embryo Cells (FEA).

Two stocks of feline gamma interferon were prepared by infection of insect cells (Sf9) infected with recombinant baculovirus containing the feline gamma interferon gene. These stocks designated DA1 and DA2 were harvested on day 5 following infection with recombinant baculovirus.

A stock of feline calicivirus was prepared in FEA cells and the titre determined by plaque assay on FEA cells. In parallel, a working bank and master bank of FEA cells were grown in tissue culture and subsequently stored under liquid nitrogen.

In a 24 well plate system, FEA cells were harvested and counted from tissue culture flasks. Wells were assigned cell controls and virus controls. The cells were used to seed 24 wells at a concentration of 2×10^5 cells/well in 1ml of medium (Dulbecos MEM/10%FCS/glutamine/NaHCO3/penecillin/streptomycin and they were allowed to incubate for 24 hours at 37°C/5% CO_2. Following the incubation step the cells were examined microscopically to ensure that they had formed a sub-confluent

monolayer. Dilutions of the Sf9 medium were added to the appropriate wells and the cells allowed to incubate at 37°C for a further 24 hours. The control wells were incubated with medium alone or wild-type baculovirus infected Sf9 cells. Following incubation, the challange calicivirus was added to the medium overlaying the cells and the cells were incubated for a further 1 hour at 37°C/5%CO_2. The concentration of virus added was sufficient to cause 100% cytophatic effect CPE, as determined when the virus was titrated, and, in this experiment, both 1PFU and 4PFU per cell were used. Following the 1 hour incubation step, the medium was removed and replaced with fresh medium. The cells were examined daily until maximum cytopathic effect was seen in the virus controls (this was 48 hours). The medium was then removed and the cell layer stained using crystal violet-formalin. The dye in the stained wells was eluted and the optical density at 595nm was measured spectrophotometrically. The results of this are represented graphically in Figure 2 and show a sharp cut-off between protection and non-protection, using dilutions of feline γ-interferon.

EXAMPLE 6

Cytopathic Effect Reduction (CPER) Assay using Vesicular Stomatitis Virus.

The VSV mutant used in this assay was designated VSVtsE2 and is derived from the New Jersey strain of VSV. This mutant strain contains two independent mutations within the NS gene and is incapabl of producing diseases in domestic livestock.

The assay procedure was essentially the same as that described for the calici virus assay. The optimum temperature for virus replication is 31°C and, initially, FEA cells were cultured at this temperature to ensure that they would still grow. The cells appeared to double normally and were subsequently infected with the VSV mutant at a rate of 0.1 PFU/cell to amplify virus stocks. The stock of virus was subsequently titrated using a standard plaque assay procedure. The 24 well plate assay system was set up as described for the calici virus assay. The only differences being that, after incubation with feline γ-interferon dilutions of DA1, the cells were challanged with VSVtsE2 at a rate of 4PFU/cell and incubation was carried out at 31°C/5%CO_2 in a category II laboratory.

Subsequently, the assay procedure was repeated using a 96 well format for the samples DA1 and DA2. The assay procedure for the 96 well format was essentially identical to the 24 well system. The only difference being that the cells were added to the wells at a concentration of $2x10^4$ cells/well in 100µl of medium. VSV virus was added at a concentration of 4pfu/cell and the assay was carried out in triplicate. Following staining, the dye was eluted from the wells and the optical density at 595nm was measured spectrophotimetrically.

The mean optical density measurements of the 96 well VSV assays performed for DA1 and DA2 are shown graphically in Figure 3. The maximum O.D measurement shown was obtained from the cell controls and the minimum value was obtained from the virus controls. For both sampl s, DA1 and DA2, there is a

gradual cut off between protection and non-protection with more points on the rectilinear portion of the graph than in the calici virus assay. For the sample DA1 the dilution which gave 50% protection, defined in terms of dye release, was found to be 1:8583 and, for DA2, the value was 1:1986. Thus, for sample DA1 and using this assay protocol, the concentration of interferon, in terms of laboratory reference units, was 8583 units/ml.

EXAMPLE 7

MHC Class II Antigen Induction Assay.

This assay was used to demonstrate that the baculovirus derived recombinant feline interferon gamma, was able to up-regulate MHC class II antigens on the feline continuous T cell line, designated F422.

Initially F422 cells were grown in tissue culture and subsequently centrifuged, washed and resuspended in complete medium to a concentration of $3x10^5$ cells/ml. Two fold serial dilutions of the interferon preparation DA1 were made in 12 well tissue culture plates. Wells were also assigned virus and cell controls. 1ml of the cell suspension was added to each well and the cells were allowed to incubate at 37°C/5%CO_2 for around 48 hours.

Following incubation, the cells in each well were divided in two to give a positive (+) and negative (-) for the subsequent flow cytometry analysis. The "positive" cells were labelled with MHC class II anti-cat monoclonal antibodies and the "negative" cells were labelled with CD8 anti-cat monoclonal

antibodi s. The MHC class II antigen is inducible on F422 cell lines whereas the cells are CD8 negative.

Following incubation, the cells were transferred to labelled 5ml falcon tubes. To each tube was added 2ml of PBS/0.1%BSA/0.01% Sodium azide to wash the cells. The tubes were then centrifuged at 1500 rpm for 5minutes at 4°C and the supernatant discarded. 50µl of MHC class II monoclonal antibody was then added to the (+) cells and 50µl of CD8 monoclonal antibody was added to the (-) cells. The cells were incubated on ice for 15 minutes. Following incubation, the cells were washed again using 2ml PBS/BSA/azide, centrifuged and the supernatant discarded. 50µl of FITC antibody was then added to each tube and the tubes allowed to incubate on ice for 15 minutes. The cells were then washed with 2ml of PBS/BSA/azide, centrifuged and the supernatant discarded. Subsequently, cell analysis was carried out using flow cytometry, or the cells were fixed using 1% paraformaldehyde and analysed at a later stage.

The MHC Class II induction assay was performed on the baculovirus interferon preparation designated DA1. This is the same preparation that had been used in the VSV assay described above. The results of the flow cytometry analysis is given in Figures 4A to 4D. The gate labelled H in the figures represents background fluorescence, while the gate labelled L represents the percentage of cells which are demonstrating MHC Class II up-regulation. The anti-cat MHC Class II monoclonal antibody was used to demonstrate any changes in MHC Class II expression on the cell line. The anti-cat CD8 monoclonal

antibody was used to measure non-specific binding of mouse monoclonal antibody. The cell controls had been incubated in the absence of interferon-gamma. The virus controls were cells which had been incubated with Sf9 cell supernatants containing wild-type PAK6 baculovirus at a 1:32 dilution. Both of these controls were labelled with anti-cat MHC class II and anti-cat CD8 monoclonal antibodies in the same way as the cells incubated with the interferon preparations. The results of the flow cytometry analysis are shown in Table 1 and in Figures 4A to 4D. The assay procedure was carried out in duplicate and the figures shown in Table 1 are mean values. All of the cells which were labelled with the anti-cat CD8 monoclonal antibody showed negligible fluorescence (see Figure 4B and Table 1).

Untreated F422 cells demonstrate low level class II expression (see Figure 4A). However, incubation of the cells with interferon dilutions clearly enhances the expression of MHC class II on these cells. This is shown by the marked shift in fluorescence as shown by comparing Figures 4A with 4C and 4D. Figure 4C shows the result of cells incubated with 1:32 dilution of interferon and Figure 4D shows the result of cells incubated with a 1:4096 dilution of interferon. At high concentrations of interferon (1:4 to 1:32), while MHC Class II was still up-regulated, the degree of up-regulation was less than for those cell incubated with lower dilutions. The MHC Class II induction assay seem to be very sensitive and up-regulation is still seen at dilutions of interferon DA1 of 1:4096.

TABLE 3

Flow cytometry of F422 cells following IFN-γ

DILUTION	% fluorescence MHC II	% fluorescence CD8
1:4	31.1	negligible
1:8	38.9	negligible
1:16	44.9	negligible
1:32	63.55	negligible
1:64	69.9	negligible
1:128	68.85	negligible
1:256	69.3	negligible
1:512	66.95	negligible
1:1024	67.6	negligible
1:2048	70.55	negligible
1:4096	71.6	negligible
cell control	1.1	0.7
virus control	1.75	0.5

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CLAIMS

1. A polynucleotide fragment encoding feline γ-interferon.

2. A polynucleotide fragment according to claim 1 characterised in that said polynucleotide fragment encodes a polypeptide having an amino acid sequence shown in Figure 1 or a derivative thereof.

3. A polynucleotide fragment according to claim 1 characterised in that it is a polynucleotide fragment which is substantially the same as the polynucleotide fragment shown in Figure 1 or a derivative thereof.

4. A recombinant nucleic acid molecule comprising a polynucleotide fragment according to any one of claims 1 to 3.

5. A recombinant nucleic acid molecule according to claim 4 characterised in that the recombinant nucleic acid molecule comprises regulatory control sequences operably linked to said polynucleotide fragment for controlling expression of said polynucleotide fragment.

6. A recombinant nucleic acid molecule according to any one of claims 4 or 5 wherein the recombinant nucleic acid molecule is a plasmid.

7. A recombinant nucleic acid molecule according to any one

of claims 4 or 5 wherein the recombinant nucleic acid molecule is derived from a viral vector.

8. A prokaryotic or eukaryotic host cell transformed, by a polynucleotide fragment or recombinant molecule according to any one of the preceding claims.

9. A recombinant feline γ-interferon polypeptide or derivative thereof displaying substantially the same biological activity as naturally occurring feline γ-interferon.

10. A recombinant feline γ-interferon polypeptide as shown in Figure 1 or derivatives thereof.

11. An antibody immuno-reactive with a polypeptide or fragment according to claims 9 or 10.

12. A polynucleotide fragment according to any one of claims 1-3 for use in therapy.

13. A recombinant nucleic acid molecule according to any of claims 4 to 6 for use in therapy.

14. A recombinant polypeptide or derivative thereof according to claims 9 or 10 for use in therapy.

15. A pharmaceutical composition comprising a polynucleotide fragment according to any of claims 1 to 3.

16. A pharmaceutical composition comprising a polypeptide or derivative thereof according to claims 9 or 10.

```
CTACTGATTTCAACTTCTTTGGCCTAACTCTCCGAAACGATGAATTACACAAGTTTTATT
                        *  L  S  E  T  M  N  Y  T  S  F  I

TTCGCTTTCCAGCTTTGCATAATTTTGTGTTCTTCTGGTTATTACTGTCAGGCCATGTTT
F  A  F  Q  L  C  I  I  L  C  S  S  G  Y  Y  C  Q  A  M  F

TTTAAAGAAATAGAAGAGCTAATGGGATATTTTAATGCAAGTAATCCAGATGTAGCAGAT
F  K  E  I  E  E  L  M  G  Y  F  N  A  S  N  P  D  V  A  D

GGTGGGTCGCTTTTCGTAGACATTTTGAAGAACTGGAAAGAGGAGAGTGATAAAACAATA
G  G  S  L  F  V  D  I  L  K  N  W  K  E  E  S  D  K  T  I

ATTCAAAGCCAAATTGTCTCCTTCTACCTGAAAATGTTTGAAAACCTGAAAGATGATGAC
I  Q  S  Q  I  V  S  F  Y  L  K  M  F  E  N  L  K  D  D  D

CAGCGCATTCAAAGGAGCATGGACACCATCAAGGAAGACATGCTTGATAAGTTGTTAAAT
Q  R  I  Q  R  S  M  D  T  I  K  E  D  M  L  D  K  L  L  N

ACCAGCTCCAGTAAACGGGATGACTTCCTCAAGCTGATTCAAATCCCTGTGAATGATCTG
T  S  S  S  K  R  D  D  F  L  K  L  I  Q  I  P  V  N  D  L

CAGGTCCAGCGCAAAGCAATAAATGAACTCTTCAAAGTGATGAATGATCTCTCACCAAGA
Q  V  Q  R  K  A  I  N  E  L  F  K  V  M  N  D  L  S  P  R

TCTAACCTGAGGAAGCGGAAAAGGAGCCAGAATCTGTTTCGAGGCCGTAGAGCATCGAAA
S  N  L  R  K  R  K  R  S  Q  N  L  F  R  G  R  R  A  S  K

TAATGGTTGTCCTGCCTGCAATATTTG
*
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FIG. 1

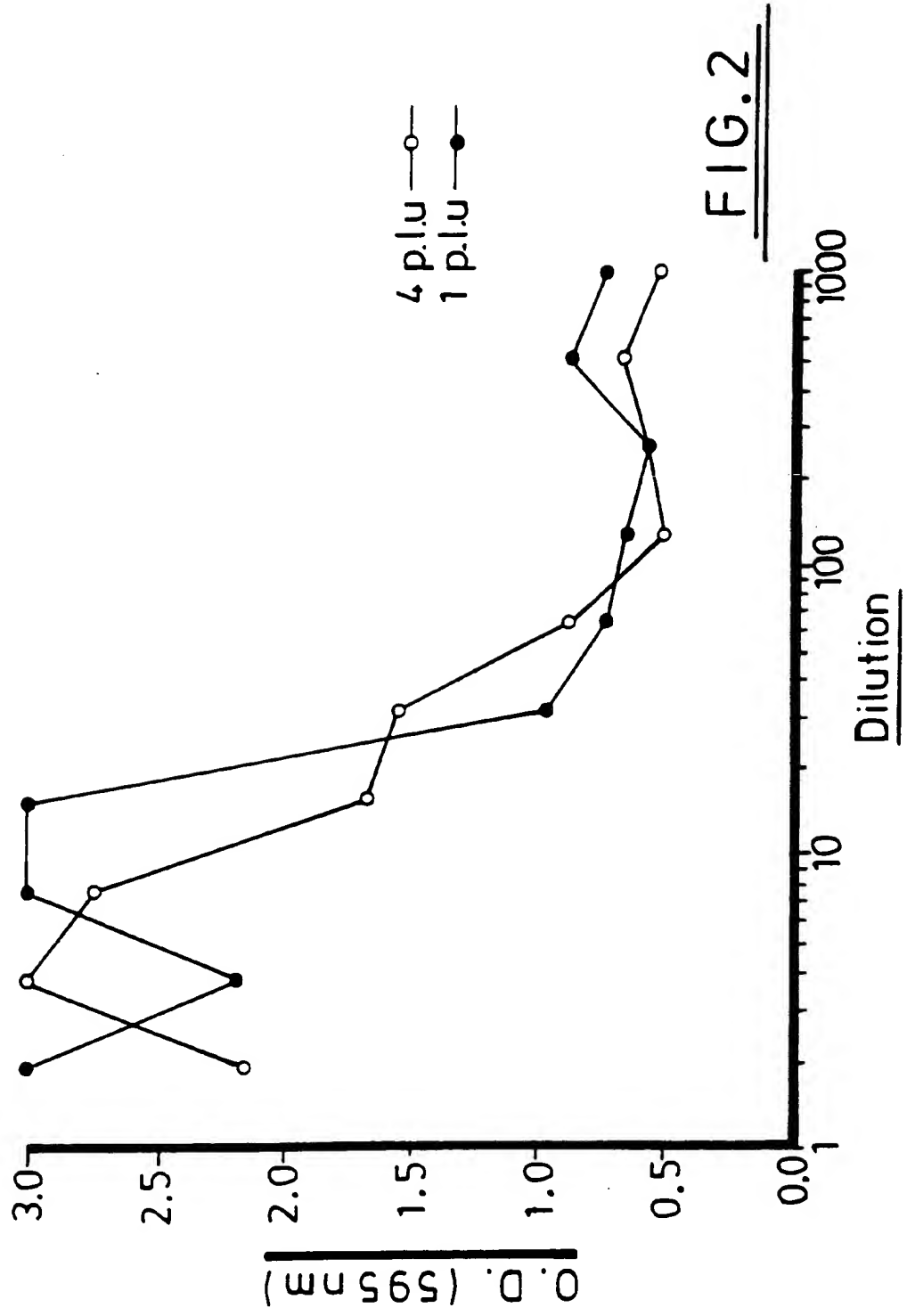

FIG.2

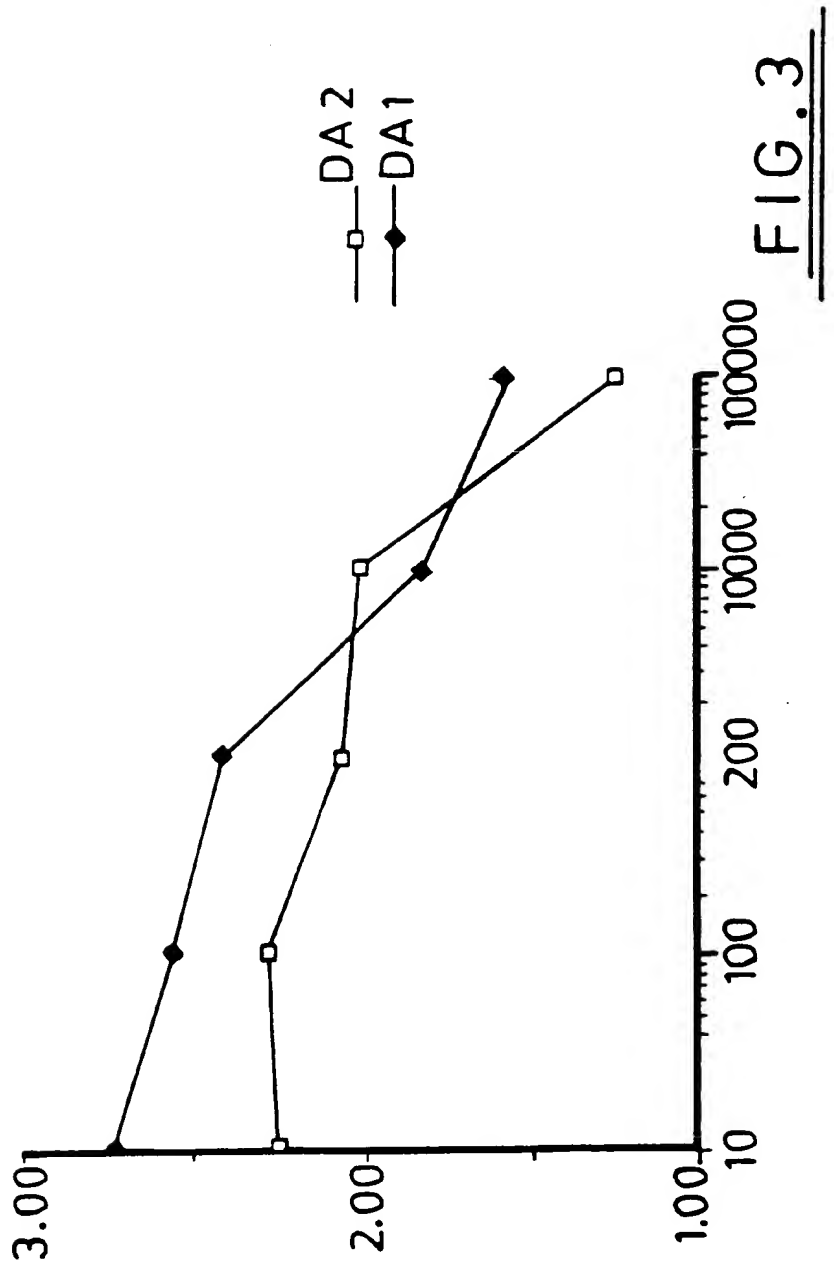

FIG. 3

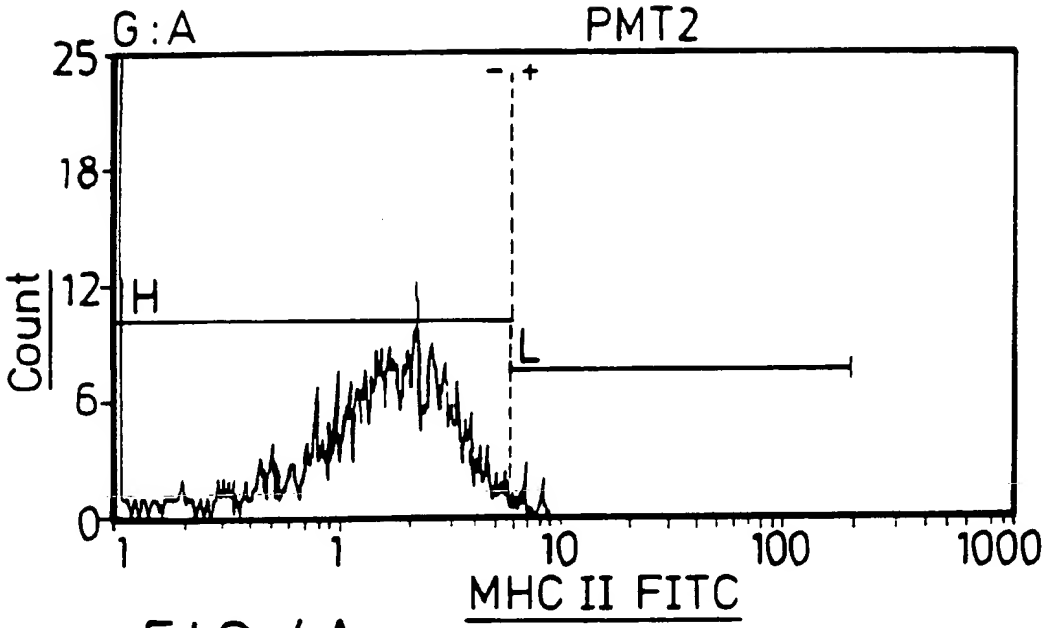
G:A
PMT2
25
18
12
6
0
Count
H
L
1
1
10
100
1000
MHC II FITC

FIG.4A

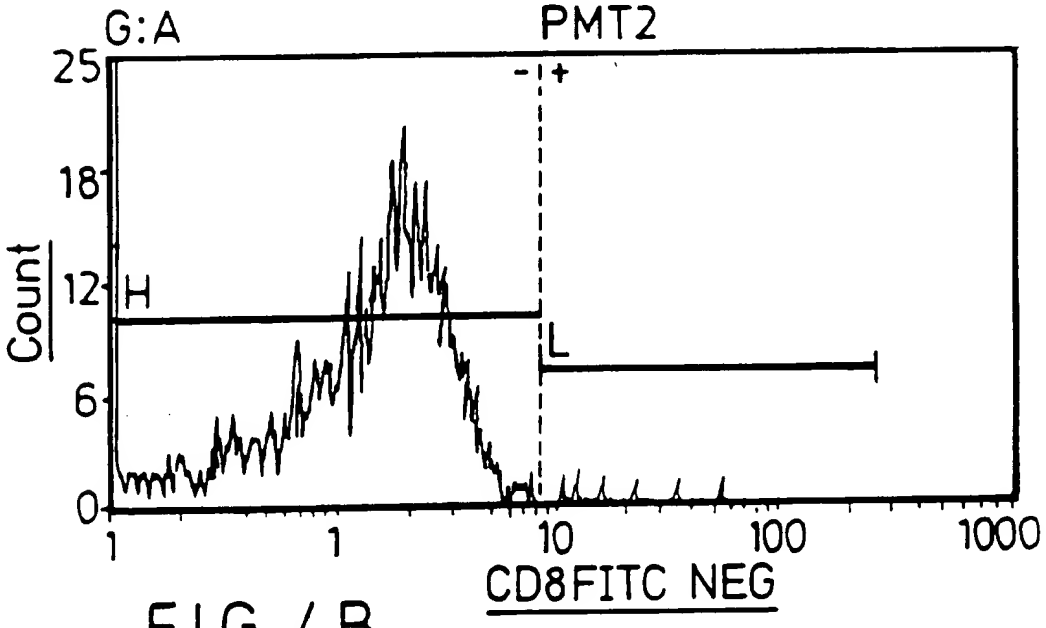
G:A
PMT2
25
18
12
6
0
Count
H
L
1
1
10
100
1000
CD8FITC NEG

FIG.4B

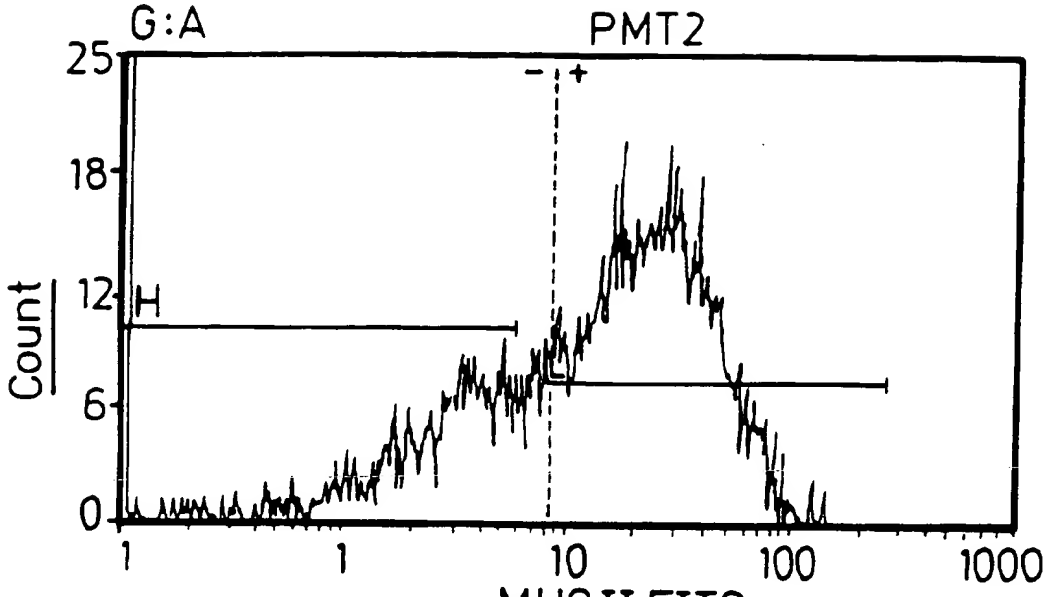
G:A
PMT2
25
18
12
6
0
Count
H
1
1
10
100
1000
MHC II FITC

FIG. 4C

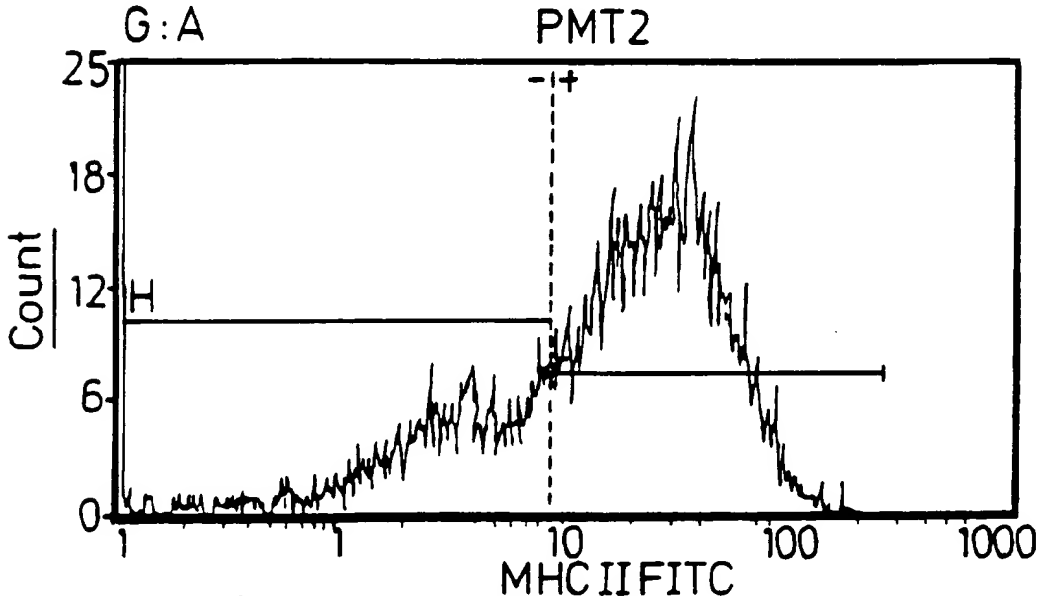
G:A
PMT2
25
18
12
6
0
Count
H
1
1
10
100
1000
MHC II FITC

FIG. 4D